

REMARKS

Claims 1-42, 44-53-66, and 70-76 are currently under Final rejection. Claims 2-3, 6-11, 20, 22-23, 26-31, 40, 43-55, 58, 60-88, some of which were withdrawn previously, are canceled herein. Applicants reserve the right to prosecute any canceled subject matter at a later date or in a timely filed divisional application.

Claims 1, 4, 5, 12, 21, 24, 25, 32, 34, 41, 42, 56, and 57 have been amended. Support for the amendments can be found throughout the specification as filed originally, for example at page 20, line 30 et seq.; page 4, line 34 et seq.; Figure 4; Figure 5; Example 7, Example 8. New claims 60-62 have been added. Support for the new claims can be found throughout the application as filed originally, for example at page 5, lines 11-27, page 6, lines 26-29, page 11, lines 6-11, Examples 1, 8, 10, 11, and 13. No new matter is introduced by these amendments.

Objections and Rejections under 35 U.S.C. § 121(c)

On page 2 of the Final Office Action mailed October 30, 2007, the Examiner objected to claims 3, 23, and 45, 47-53 and 55 as “contain[ing] the wrong identifier.” On page 3, the Examiner maintained the rejection of claims 3, 23, and 45, as listing non-elected species. These claims have been canceled, hence these objections are moot and may be withdrawn.

Rejections under 35 U.S.C. § 103(a)

The Examiner, on page 4 of the final Office Action maintains the rejection of claims 1-42, 59-66, and 70-76 “as being unpatentable over U.S. Patent No. 5,741,899 ... Capon et al., in view of Spencer et al. (1996) Current Biology, Vol. 6(7), 839-847 and Blau et al. (1996) Blood, vol. 88 (10 Suppl. 1 part 1-2), p542A, meeting abstract” Applicants traverse the rejection.

The claimed invention relates to methods for rendering a subpopulation of mammalian primary hematopoietic stem cells susceptible to divalent ligand-induced growth, proliferation or differentiation, which method comprises transducing one or more cells of a population of primary mammalian hematopoietic stem cells with at least one retroviral vector comprising at least one recombinant DNA construct encoding a fusion protein which comprises at least one signaling domain derived from a thrombopoietin receptor and at least one ligand-binding domain

derived from F36V, which is heterologous with respect to the signaling domain and binds to a divalent ligand drug, and exposing the transduced cells to a concentration of a particular drug class of divalent ligands capable of inducing association between two or more molecules of F36V (such as AP1903, AP1510, or AP20187) effective to induce association of two or more of the encoded fusion proteins, whereupon growth, proliferation or differentiation of said cells is induced; wherein the transduction is carried out *in vivo* or after the cells have been removed from the mammal from which the cells originated, and wherein said transduced cells are suitable for introduction into a mammal.

The claimed invention thus provides for pharmacologically-responsive fusion proteins that are useful for specifically and reversibly expanding genetically modified primary hematopoietic stem cell populations. This allows, for the first time, application of a neutral, targeted approach that does not rely on either negative selective pressure (e.g., antibiotic resistance) or addition of a growth factor that might have systemic implications, to provide selective positive inducement for cell growth, proliferation and/or differentiation.

Returning to the cited combination of references, on page 5 of the Final Office Action the Examiner clarifies the position that “Capon et al. was relied upon for the teaching that the extracellular or intracellular inducer-responsive clustering domain of the chimeric protein is derived from immunophilin, e.g., FKBP, and that the cytoplasmic signal transduction domain is derived from homodimerizing receptors such as G-CSFR, EPO-R, GHR, PRLR, TPOR, and gp130 (Capon et al., columns 7, 9, 13, 14, 34-35, and 42-43”

In doing so, the Examiner has focused on only a few of the myriad of domains identified in Capon. For example, regarding the inducer-responsive clustering domain, Capon recites, *inter alia*:

The intracellular clustering domain (ICD) can be obtained from the inducer binding domains of a variety of intracellular proteins. For example, eukaryotic steroid receptor molecules can be used as ICDs (e.g. the receptors for estrogen, progesterone, androgens, glucocorticoids, thyroid hormone, vitamin D, retinoic acid, 9-cis retinoic acid and ecdysone). In addition, variants of steroid and other receptors which fail to bind their native inducer, but still bind to an antagonist, can be prepared by one skilled in the art and used to make the CPRs of this invention. For example, a C-terminal deletion mutant of the human progesterone receptor, which fails to bind progesterone, can be clustered by the addition of progesterone antagonists, including RU 486. Binding domains from the eukaryotic immunophilin family of molecules may also be used as ICDs. Examples include but are not limited to members of the cyclophilin family: mammalian cyclophilin A, B and C, yeast cyclophilins 1 and 2, *Drosophila* cyclophilin analogs such as ninaA; and members of the FKPB family: the various mammalian isoforms of FKBP and the FKBP

analog from Neurospora. For example, the inducer binding portion of the immunophilin, FKBP12, which can be clustered in the cytoplasm by the addition of FK1012, a synthetic dimeric form of the immunosuppressant FK506 can be used as an ICD. Col. 14, line 55-col. 14, line 16 (internal citations omitted).

In this paragraph alone, Capon refers to at least twenty domains, including receptors for estrogen, progesterone, androgens, glucocorticoids, thyroid hormone, vitamin D, retinoic acid, 9-cis retinoic acid and ecdysone, a C-terminal deletion mutant of the human progesterone receptor, mammalian cyclophilin A, cyclophilin B and cyclophilin C, yeast cyclophilins 1 and 2, *Drosophila* cyclophilin analogs such as ninaA, various mammalian isoforms of FKBP and the FKBP analog from Neurospora, and FKBP12.

Regarding the cytoplasmic signal transduction domain, Capon recites, *inter alia*:

The proliferation signaling domains (PSDs) that comprise the chimeric proliferation receptors (CPRs) of the present invention (both CIPRs and CEPRs) may be obtained from the cytoplasmic signal-transducing domains of the cytokine/hematopoietin receptor superfamily. The members of this mammalian receptor superfamily can transduce proliferative signals in a wide variety of cell types. These receptors are structurally related to each other. The cytoplasmic domains of the signal-transducing subunits may contain conserved motifs that are critical for transduction of proliferative signals. In contrast to the growth factor receptors previously described in chimeric receptors, the cytoplasmic portions of the cytokine receptor superfamily proteins that comprise the PSDs employed in the present invention do not contain any kinase domains or other sequences with recognizable catalytic function. Further, although the growth factor receptors described by Ullrich and the cytokine receptors employed in the present invention both dimerize upon binding of inducer, the dimerized growth factor receptors activate their intrinsic receptor kinase activity, while the dimerized cytokine receptors employed in the present invention stimulate the activity of associated tyrosine kinases. The signal-transducing components of the cytokine receptors to be used in the PSDs of the present invention include, but are not limited to, Interleukin-2 receptor β (IL-2R β), IL-2R γ , IL-3R β , IL-4R, IL-5R α , IL-5R β , IL-6R, IL-6R, gp130, IL-7R, IL-9R, IL-12R, IL-13R, IL-15R, EPO-R (erythropoietin receptor), G-CSFR (granulocyte colony stimulating factor receptor), GM-CSFR α (granulocyte macrophage colony stimulating factor receptor α) GM-CSFR β , LIFR α (leukemia inhibitory factor receptor α), GHR (growth hormone receptor), PRLR (prolactin receptor), CNTFR (ciliary neurotrophic factor receptor), OSMR (oncostatin M receptor) IFN α/β (interferon α/β receptor), IFN γ , TFR (tissue factor receptor), and TPOR (thrombopoietin or mpl-ligand receptor). Col. 8, line 40-col. 9, line 20 (internal citations omitted.)

Column 9, to which the Examiner refers, recites at least IL-2R β , IL-2R γ , IL-3R β , IL-4R, IL-5R α , IL-5R β , IL-6R, gp130, IL-7R, IL-9R, IL-12R, IL-13R, IL-15R, EPO-R, G-CSFR, GM-CSFR α , GM-CSFR β , LIFR α , GHR, PRLR, CNTFR, OSMR, IFN α/β , IFN γ , TFR, and TPOR, providing at least twenty-six proliferation domains to the list of possible domains. Cols. 34-35

and 42-43, to which the Examiner refers, add numerous JAK receptors.

In just these two presented paragraphs, Capon presents over **500** possible chimeric fusion proteins, providing no expectation that progesterone receptor fused with gp130 would be any more successful than ninaA fused with OSMR in rendering a subpopulation of mammalian hematopoietic stem cells susceptible to drug-induced growth, proliferation or differentiation. The Capon Examples refer to FKBP only in the context of JAK or IL-2, and they refer to neither the F36V, the EpoR, nor the divalent ligand of the claimed invention.

The Examiner has cited *In re O'Farrell*, which is instructive on the present rejection:

The admonition that “obvious to try” is not the standard has been directed mainly at two kinds of error. In some cases, *what would have been “obvious to try” would have been to vary all parameters or to try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many choices were likely to be successful.* ... In others what was “obvious to try” was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave *only general guidance* as to the *particular form* of the claimed invention or how to achieve it. *In re O'Farrell*, 7 USPQ2d 1673 (Fed. Cir. 1988) (emphasis added).

In that regard, the Examiner’s apparent selection of but a few of Capon’s parameters and numerous possible choices, in total disregard of the numerous others suggested equally by Capon can be characterized as “obvious to try” or hindsight.

Spencer and Blau provide two further constructs, neither of which read on the pending claims. More specifically, on page 5 of the Action, the Examiner asserts that “Blau et al. provides specific evidence that dimerization of chimeric receptors comprising FKBP and EpoR leads to cell proliferation.” Additionally, on page 6 of the Action, the Examiner explains that “Spencer was relied upon for teaching specific concentrations of FK1012 which induce dimerization of chimeric proteins expressed by T cells comprising FKBP domains and Fas receptor and to methods to determine the optimal concentration of FK1012 to induce the dimerization of chimeric proteins comprising FKBP and Fas receptor.”

Even if Spencer and Blau supplement Capon by referring to particular constructs, these are not the constructs according to the method of the present claims. In other words, the combination of Capon, Spencer, and Blau does not provide a reasonable expectation of success regarding the particular form included in the claimed invention: at least one signaling domain derived from a thrombopoietin receptor and at least one drug-binding domain derived from F36V, in the context of rendering a subpopulation of mammalian primary hematopoietic stem cells susceptible to

divalent ligand-induced growth, proliferation or differentiation by transducing primary mammalian hematopoietic stem cells with at least one retroviral vector comprising a recombinant DNA encoding a signaling domain derived from a thrombopoietin receptor fused to an F36V, then exposing the transduced cells to the particular class of claimed divalent ligand effective to induce association of two or more of the encoded fusion proteins, such that growth, proliferation or differentiation of said cells is induced, wherein the transduction is carried out *in vivo* or after the cells have been removed from the mammal from which the cells originated, and wherein said transduced cells are suitable for introduction into a mammal.

Moreover, the transduction of the primary hematopoietic stem cells with the chimeric receptor requires, according to the claimed methods, is followed by exposure to a concentration of the drug AP20187 effective to induce association of two or more fusion proteins, thereby inducing growth, proliferation or differentiation of said cells.

In that regard, Capon presents a myriad of inducer drugs, for example, *inter alia*:

Examples of inducers include, but are not limited to synthetic dimeric molecules such as FK1012 or dimeric derivatives of the binding domains of other immunophilin binding molecules such as cyclosporin, rapamycin and 506BD. Steroids, such as estrogen, progesterone, the androgens, glucocorticoids, thyroid hormone, vitamin D, retinoic acid, 9-cis retinoic acid or ecdysone, or antagonists or derivatives of these molecules may also be used as intracellular inducer molecules. In particular the steroid antagonist RU 486 may be used. Col. 15, lines 24-35 (internal citations omitted).

Extracellular inducers of the present invention can be antigens which bind the ECDs, described above. These may include viral proteins, (e.g. gp120 and gp41 envelope proteins of HIV, envelope proteins from the Hepatitis B and C viruses, the gB and other envelope glycoproteins of human cytomegalovirus, the envelope proteins from the Kaposi's sarcoma-associated herpesvirus), and surface proteins found on cancer cells in a specific or amplified fashion, (eg the IL-14 receptor, CD19 and CD20 for B cell lymphoma, the Lewis Y and CEA antigens for a variety of carcinomas, the Tag72 antigen for breast and colorectal cancer, EGF-R for lung cancer, and the HER-2 protein which is often amplified in human breast and ovarian carcinomas). For other receptors, the receptors and ligands of particular interest are CD4, where the ligand is the HIV gp120 envelope glycoprotein, and other viral receptors, for example ICAM, which is the receptor for the human rhinovirus, and the related receptor molecule for poliovirus. Col. 14, lines 37-54 (internal citations omitted).

The Examiner "supplements" Capon's host of inducers with Spencer, noting on page 6 of the Action that "the essential teaching of Spencer is that FK1012 can be effectively used as a synthetic inducer ... and that the determination of concentrations of FK1012 capable of inducing

dimerization was routine." The Examiner continues that "[Because] Blau et al. does not teach what concentration of FK1012 was used in their experiments that successfully induced dimerization and signaling leading to cell growth, the methods disclosed in Spencer simply provide the reasonable expectation that it would be routine to determine such an effective concentration of FK1012 as used in Blau et al." These references, in combination, do not mention much less suggest the use of the divalent ligand of in the claimed invention.

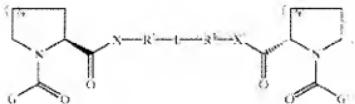
The Examiner argues, on page 7, that because the prior art combination did not raise concerns about the difficulties with using FK1012, one skilled in the art would have a reasonable expectation that FK1012 would succeed. The instant specification, which clearly can not be ignored as a source of expectation of success for one of ordinary skill, presents at least two additional problems with FK1012:

Two factors limit the use of FK1012 ... for *in vivo* studies. First, FK1012 ...[is] available only in limited quantities: [it] cannot be purchased, and must be chemically synthesized. Second, the *in vivo* half life of FK1012 is less than one hour, severely impeding efforts to extend *in vitro* observations to the *in vivo* setting. Page 37, lines 8-12.

* * *

AP20187 has two major advantages relative to FK1012: 1) AP20187 does not bind to endogenous FKBP12, and in many situations the drug is effective at lower concentrations than are required for FK1012; and 2) The *in vivo* half life of AP20187 is 9 hours, greatly improving the feasibility of *in vivo* studies. Page 45, line 35-page 46, line 3.

The Examiner noted, on page 7 of the Action, that "the instant claims encompass the use of FK1012 and are not limited to an embodiment ... referred to as being less toxic by applicants." The claims as amended herein do not encompass the use of FK1012, but recite the use of the less-toxic



(which includes AP20187, AP1903, and AP1510), which is not suggested by any combination of the cited art.

On page 8 of the Action, the Examiner states that "the claims as amended are no longer limited to 'primary cells' but rather recite 'hematopoietic stem cells' which encompass any

hematopoietic stem cell, whether primary or established as a cell line." The claims had been amended pursuant to a restriction requirement, but have now been amended herein to recite "primary mammalian hematopoietic stem cells."

It was clear to those of ordinary skill in the art at the time the instant application was filed that established cell lines would not always serve as an adequate indicator of *in vivo* activity. In particular, experiments related to growth, proliferation and differentiation in immortalized long-term cultured cells might yield results not applicable to primary cells. To wit, standard text books of the time warned that:

Cells that have ... become **established** to form a ... cell line ... can be perpetuated indefinitely, but their properties have changed in passing ... and may indeed continue to change during the adaptation to culture. These changes may partly resemble those involved in tumor formation, which reduces the usefulness of the cells. ... [A]ny established cell line provides only an approximation of *in vivo* control. The need for **caution** in analyzing the genetic basis for **growth control** in such lines is emphasized by the fact that almost always they suffer changes in chromosome complement ... Lewin, Genes VI at 1131-33 (Oxford Univ. Press, Inc., New York, 1997) (second and third emphases added) (excerpt provided herewith).

Moreover, the claimed invention provides for cells that are suitable for introduction into a mammal. Clearly in the context of the claims, the cited art provides little guidance regarding primary mammalian hematopoietic stem cells suitable for introduction into a mammal. More specifically, the cell lines of Spencer and Blau would not provide for primary mammalian hematopoietic stem cells suitable for introduction into a mammal. For example, established cell lines are often oncogenic, precluding their use in clinical settings, and within the context of the pending claims rendering them unsuitable for introduction into a mammal. It is well known in the art that Jurkat T-cells are human leukemia cells. Gill & Watson, 152(6) J. Exp. Med. 1709-19 (1980) (Abstract supplied herewith). Moreover, Spencer used these cells only *in vitro* and only in the context of programmed death, there is no suggestion in the combined references that Jurkat cells would be suitable for cell differentiation or expansion, and certainly not for treating disease as recited in, e.g., claims 56 or 59. Finally, the cell line of Spencer was used with a drug that could not be used in conjunction with cells suitable for introduction into a mammal because of the difficulties of FK1012 evidenced in the instant application (e.g., FK1012 binds to endogenous FKBP as discussed above).

Similarly, Blau refers to *in vitro* studies using the murine IL3-dependant Ba/F3 cell line.

As noted in earlier Replies of record, an IL-3 dependant cell line, in the context of the pending claims, would not be suitable for introduction into a mammal, particularly a human mammal.

The Examiner, on page 8, states that "Capon et al. clearly teaches *in vivo* methods." Capon refers to all sorts of methods for numerous cell types. For example, engineered T-cells expressing dual chimera receptors to be activated at disease site (col. 18, lines 9-12); cytotoxic T-cells that recognize HIV antigens (col. 18, lines 15-19); lymphocytes targeting neoplastic cells, virus-infected cells, parasite-infected cells, or any other diseased cells (col. 20, lines 16-21); cytotoxic CD8⁺ T-cells against CMV, HIV, Hepatitis B virus, Hepatitis C virus, Kaposi's sarcoma-associated Herpes virus, Herpes Simplex virus, Herpes Zoster virus, and papilloma viruses, neoplastic cells, IL-14 receptor, CD19 and CD20 for B cell lymphoma, the Lewis Y and CEA antigens for a variety of carcinomas, Tag72 antigen for breast and colorectal cancer, EGF-R for lung cancer, human Heregulin (Hrg), or against autoimmune cells in the treatment of autoimmune diseases such as Systemic Lupus Erythematosis (SLE), myasthenia gravis, diabetes, rheumatoid arthritis, and Grave's disease (col. 20, lines 22-60); CD4⁺ helper T-cells against cancer cells and mycobacterial infections, including *Mycobacterium avium*, *Mycobacterium tuberculosis* and *Mycobactium leprae* (col. 20, lines 61-67); "various cell types" to reconstruct existing tissue or provide new tissue in transplantation therapy, for example, keratinocytes for skin (col. 21, lines 1-15); islets of Langerhans, "immune cells," myoblasts, hepatocytes, endothelial cells, nerve cells (col. 21, lines 16-35); and "additional cell types" to correct gene defects or produce proteins (col. 21, lines 36-54).

Regarding stem cells, Capon does not refer to primary hematopoietic stems cells of the claimed invention, but recites hematopoietic stems in the context of a laundry list including:

[A] wide variety of target host cells, normally cells from vertebrates, more particularly, mammals, desirably domestic animals or primates, particularly humans ... the subject invention may also find application in the expansion of lymphoid cells, e.g., T lymphocytes, B lymphocytes, cytotoxic lymphocytes (CTL), natural killer cells (NK), tumor-infiltrating-lymphocytes (TIL) or other cells which are capable of killing target cells when activated ... hematopoietic stem cells, which develop into cytotoxic effector cells with both myeloid and lymphoid phenotype including granulocytes, mast cells, basophils, macrophages, natural killer (NK) cells and T and B lymphocytes... diseased cells, such as cells infected with HIV, HTLV-I or II, cytomegalovirus, hepatitis B or C virus, *Mycobacterium avium*, etc., neoplastic cells, or autoimmune disease-causing cells where the diseased cells have a surface marker associated with the diseased state may be made specific targets of the cells ... expressing the CPRs of the present invention. Col. 16, lines 7-35.

Spencer and Blau only add additional cell types, Jurkat and Ba/F3 cells, with no mention or suggestion regarding methods for rendering a subpopulation of mammalian primary hematopoietic stem cells susceptible to divalent ligand-induced growth, proliferation or differentiation by transducing primary mammalian hematopoietic stem cells with at least one retroviral vector comprising at least one recombinant DNA construct encoding a fusion protein which comprises at least one signaling domain derived from a thrombopoietin receptor fused to at least one drug-binding domain derived from F36V and exposing the transduced cells to a concentration of the drug AP20187 effective to induce association of two or more fusion proteins, thereby inducing growth, proliferation or differentiation of said cells, wherein the transduction is carried out *in vivo* or after the cells have been removed from the mammal from which the cells originated, and wherein said transduced cells are suitable for introduction into a mammal.

In combination, the cited references do not provide an expectation of success regarding the primary hematopoietic stem cells as used in the methods of the claimed invention. Rather, these references provide numerous possible choices or present a new technology or general approach that seemed to be a promising field of experimentation, and give only general guidance as to the particular form of the claimed invention or how to achieve it. Such references do not support an obviousness rejection under the standard set forth by the court in *In re O'Farrell*, 7 USPQ2d 1673 (Fed. Cir. 1988). Moreover, given the specificities of the amended claims, combining the cited references fails to provide a “combinati[on of] previously known elements,” but rather demonstrates that the claimed invention reflects an advancement and “real innovation.” *KSR Int'l Co v. Teleflex Inc.*, No. 04-1350 (April 30, 2007) at 14, 15. Hence, Applicants respectfully request that this rejection be withdrawn.

The Examiner, on page 7 of the Office Action, rejects claims 44-53 and 55-58 under 35 U.S.C. § 103 “as being unpatentable over … Capon et al., in view of U.S. Patent No. 5,994,313 (11/30/99), hereafter referred to as Crabtree et al., Spencer … and Blau (1996).” Applicants traverse the rejection.

Claims 44-55 and 58 have been canceled. Regarding claims 56 and 57, they recite:

56. A method for treating or preventing a hemopoietic disease or pathological condition in a mammal, comprising introducing into the mammal the bone marrow cell or cord blood cell or peripheral blood cell of Claim 4 or Claim 24.

57. The method of Claim 56 which further comprises administering to the mammal a divalent ligand having the formula:



The Examiner, on page 10, states that "Crabtree was not relied upon for teaching hematopoietic stem cells modified to express specific chimeric receptors, Capon et al. and Blau et al. were relied upon for those teachings."

The vast and various teachings of Capon, presented above, combined with Blau's techniques applied to IL-3-dependent mouse cell line, do not lead one of skill in the art to the particular form of the claimed invention of treating a hemopoietic disease or pathological condition in a mammal, comprising introducing into the mammal a bone marrow cell or cord blood cell or peripheral blood cell transduced with a retroviral vector comprising at least one recombinant DNA construct encoding a fusion protein which comprises a signaling domain derived from a thrombopoietin receptor fused to a F36V and exposing the transduced cells to the claimed divalent ligand effective to

to induce growth, proliferation or differentiation of said cells, wherein the transduction is carried out *in vivo* or after the cells have been removed from the mammal from which the cells originated, and wherein said transduced cells are suitable for introduction into a mammal, or further administering to the mammal AP20187.

The Examiner, on pages 9 and 10, states that "Capon et al. does suggest that modifications can be made to the ICD to create improved receptor-ligand binding. ... Crabtree et al. supplements Capon et al. by teaching similar chimeric proteins comprising and inducer-responsive clustering domain and a signaling domain where the inducer-responsive domain of FKBP12 contains specific amino acid changes as compare to the wild type sequences."

Capon defines modification as follows:

The term "modifications" refers to an addition of one or more amino acids to either or both of the C- and N-terminal ends of the intracellular and extracellular inducer molecules (in the case where these are proteins) or, the ECDs, ICDs, PSDs, EFSDs, or TMs, a substitution of one or more amino acids at one or more sites throughout these proteins, a deletion of one or more amino acids within or at either or both ends of these proteins, or an insertion of one or more amino acids at one or more sites in these proteins such that the inducer molecule binding to the ICD or the ECD is retained or improved as measured by binding assays known in the art, for example, Scatchard plots, or such that

the PSD, EFSD or TM domain activities are retained or improved as measured by one or more of the proliferation assays described below. In addition, modifications can be made to the intracellular and extracellular inducer molecules and to the corresponding ICDs and ECDs to create an improved receptor-ligand binding pair. Col. 4, lines 48-65.

Thus, the modifications of Capon only add further numerous permutations to the hundreds of combinations of inducers and fusion proteins recited in Capon, only a small sample of which have been presented herein.

The Examiner contends that "there is no need to modify the techniques of Crabtree, as it is the methods of Capon that are to be modified using the FKBP12 domains of Crabtree et al."

Action at page 10.

Crabtree discusses FKBP12 domains as follows:

The ligand binding ("dimerization" or "receptor") domain of any of the chimeric proteins of this invention can be any convenient domain which will allow for induction using, or bind to, a natural or unnatural ligand, preferably an unnatural synthetic ligand. The binding domain can be internal or external to the cellular membrane, depending upon the nature of the construct and the choice of ligand. A wide variety of binding proteins, including receptors, are known, including binding proteins associated with the cytoplasmic regions indicated above. Of particular interest are binding proteins for which ligands (preferably small organic ligands) are known or may be readily produced. These receptors or ligand binding domains include the FKBP_s and cyclophilin receptors, the steroid receptors, the tetracycline receptor, the other receptors indicated above, and the like, as well as "unnatural" receptors, which can be obtained from antibodies, particularly the heavy or light chain subunit, mutated sequences thereof, random amino acid sequences obtained by stochastic procedures, combinatorial syntheses, and the like. For the most part, the receptor domains will be at least about 50 amino acids, and fewer than about 350 amino acids, usually fewer than 200 amino acids, either as the natural domain or truncated active portion thereof. Preferably the binding domain will be small (<25 kDa, to allow efficient transfection in viral vectors), monomeric, nonimmunogenic, and should have synthetically accessible, cell permeable, nontoxic ligands that can be configured for dimerization.

The receptor domain can be intracellular or extracellular depending upon the design of the construct encoding the chimeric protein and the availability of an appropriate ligand. For hydrophobic ligands, the binding domain can be on either side of the membrane, but for hydrophilic ligands, particularly protein ligands, the binding domain will usually be external to the cell membrane, unless there is a transport system for internalizing the ligand in a form in which it is available for binding. For an intracellular receptor, the construct can encode a signal peptide and transmembrane domain 5' or 3' of the receptor domain sequence or by having a lipid attachment signal sequence 5' or 3' of the receptor domain sequence. Where the receptor domain is between the signal peptide and the transmembrane domain, the receptor domain will be extracellular.

The portion of the construct encoding the receptor can be subjected to mutagenesis for a variety of reasons. The mutagenized protein can provide for higher binding affinity,

allow for discrimination by the ligand of the naturally occurring receptor and the mutagenized receptor, provide opportunities to design a receptor-ligand pair, or the like. The change in the receptor can involve changes in amino acids known to be at the binding site, random mutagenesis using combinatorial techniques, where the codons for the amino acids associated with the binding site or other amino acids associated with conformational changes can be subject to mutagenesis by changing the codon(s) for the particular amino acid, either with known changes or randomly, expressing the resulting proteins in an appropriate prokaryotic host and then screening the resulting proteins for binding. Illustrative of this situation is to modify FKBP12's Phe36 to Ala and/or Asp37 to Gly or Ala to accommodate a substituent at positions 9 or 10 of FK506 or FK520. In particular, mutant FKBP12 moieties which contain Val, Ala, Gly, Met or other small amino acids in place of one or more of Tyr26, Phe36, Asp37, Tyr82 and Phe99 are of particular interest as receptor domains for FK506-type and FK520-type ligands containing modifications at C9 and/or C10.

Antibody subunits, e.g. heavy or light chain, particularly fragments, more particularly all or part of the variable region, or fusions of heavy and light chain to create high-affinity binding, can be used as the binding domain. Antibodies can be prepared against haptenic molecules which are physiologically acceptable and the individual antibody subunits screened for binding affinity. The cDNA encoding the subunits can be isolated and modified by deletion of the constant region, portions of the variable region, mutagenesis of the variable region, or the like, to obtain a binding protein domain that has the appropriate affinity for the ligand. In this way, almost any physiologically acceptable haptenic compound can be employed as the ligand or to provide an epitope for the ligand. Instead of antibody units, natural receptors can be employed, where the binding domain is known and there is a useful ligand for binding.

The ability to employ *in vitro* mutagenesis or combinatorial modifications of sequences encoding proteins allows for the production of libraries of proteins which can be screened for binding affinity for different ligands. For example, one can totally randomize a sequence of 1 to 5, 10 or more codons, at one or more sites in a DNA sequence encoding a binding protein, make an expression construct and introduce the expression construct into a unicellular microorganism, and develop a library. One can then screen the library for binding affinity to one or desirably a plurality of ligands. The best affinity sequences which are compatible with the cells into which they would be introduced can then be used as the binding domain. The ligand would be screened with the host cells to be used to determine the level of binding of the ligand to endogenous proteins. A binding profile could be defined weighting the ratio of binding affinity to the mutagenized binding domain with the binding affinity to endogenous proteins. Those ligands which have the best binding profile could then be used as the ligand. Phage display techniques, as a non-limiting example, can be used in carrying out the foregoing. Col. 22, line 54-col. 24-line 21.

Hence, it is clear that Crabtree discusses a plethora of ligand domains, of which FKBP12 domains are but a subset. Similarly, Crabtree refers to a sea of ligands, for example:

The subject compounds will for the most part have two or more units, where the units can be the same or different, joined together through a central linking group. The

"units" will be individual moieties (e.g., FK506, FK520, cyclosporin A, a steroid, etc.) capable of binding the receptor domain. Each of the units will usually be joined to the linking group through the same reactive moieties, at least in homodimers or higher order homo-oligomers.

As indicated above, there are a variety of naturally-occurring receptors for small non-proteinaceous organic molecules, which small organic molecules fulfill the above criteria, and can be dimerized at various sites to provide a ligand according to the subject invention. Substantial modifications of these compounds are permitted, so long as the binding capability is retained and with the desired specificity. Many of the compounds will be macrocyclics, e.g. macrolides. Suitable binding affinities will be reflected in Kd values well below 10^{-4} , preferably below 10^{-6} , more preferably below about 10^{-7} , although binding affinities below 10^{-9} or 10^{-10} are possible, and in some cases will be most desirable.

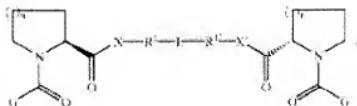
Currently preferred ligands comprise oligomers, usually dimers, of compounds capable of binding to an FKBP protein and/or to a cyclophilin protein. Such ligands includes homo- and heteromultimers (usually 2-4, more usually 2-3 units) of cyclosporin A, FK506, FK520, and rapamycin, and derivatives thereof, which retain their binding capability to the natural or mutagenized binding domain. Many derivatives of such compounds are already known, including synthetic high affinity FKBP ligands, which can be used in the practice of this invention. Sites of interest for linking of FK506 and analogs thereof include positions involving annular carbon atoms from about 17 to 24 and substituent positions bound to those annular atoms, e.g. 21 (allyl), 22, 37, 38, 39 and 40, or 32 (cyclohexyl), while the same positions except for 21 are of interest for FK520. For cyclosporin, sites of interest include MeBmt, position 3 and position 8.

Of particular interest are modifications to the ligand which change its binding characteristics, particularly with respect to the ligand's naturally occurring receptor. Concomitantly, one would change the binding protein to accommodate the change in the ligand. For example, one can modify the groups at position 9 or 10 of FK506, so as to increase their steric requirement, by replacing the hydroxyl with a group having greater steric requirements, or by modifying the carbonyl at position 10, replacing the carbonyl with a group having greater steric requirements or functionalizing the carbonyl, e.g. forming an N-substituted Schiff's base or imine, to enhance the bulk at that position. Various functionalities which can be conveniently introduced at those sites are alkyl groups to form ethers, acylamido groups, N-alkylated amines, where a 2-hydroxyethylamine can also form a 1,3-oxazoline, or the like. Generally, the substituents will be from about 1 to 6, usually 1 to 4, and more usually 1 to 3 carbon atoms, with from 1 to 3, usually 1 to 2 heteroatoms, which will usually be oxygen, sulfur, nitrogen, or the like. By using different derivatives of the basic structure, one can create different ligands with different conformational requirements for binding. By mutagenizing receptors, one can have different receptors of substantially the same sequence having different affinities for modified ligands not differing significantly in structure.

Other ligands which can be used are steroids. The steroids can be oligomerized, so that their natural biological activity is substantially diminished without loss of their binding capability with respect to a chimeric protein containing one or more steroid receptor domains. By way of non-limiting example, glucocorticoids and estrogens can be so used. Various drugs can also be used, where the drug is known to bind to a particular receptor with high affinity. This is particularly so where the binding domain of the receptor is known, thus permitting the use in chimeric proteins of the invention of only the binding domain, rather than the entire native receptor protein. For this purpose, enzymes and

enzyme inhibitors can be used. Col. 29, line 52-col. 30, line 63 (internal citations omitted).

Although Crabtree, in the Examples section, discusses the formation of different FK drugs, including four derivative FK506 molecules, six derivative FK1012 molecules (Examples 13 to 18), and several cyclosporin derivatives, Crabtree does not refer to which drug might serve as a ligand in primary hematopoietic stem cells in the context of the claimed invention. Moreover, none of the ligands or modifications thereto provide for the divalent ligand



which is, in and of itself, patentably distinct (see U.S. Patent No. 6,150,527). Nor does Crabtree suggest that this divalent ligand could be used to as a method of treating a hemopoietic disease or pathological condition in a mammal, comprising introducing into the mammal a bone marrow cell or cord blood cell or peripheral blood cell transduced with a retroviral vector comprising at least one recombinant DNA construct encoding a fusion protein which comprises at least one signaling domain derived from a thrombopoietin receptor fused to at least one drug-binding domain derived from F36V and exposing the transduced cells to a concentration of a divalent ligand capable of inducing association between two or more molecules of F36V (such as the divalent ligands AP1903, AP1510, or AP20187) effective to induce association of two or more of the encoded fusion proteins, whereupon growth, proliferation or differentiation of said cells is induced, wherein the transduction is carried out *in vivo* or after the cells have been removed from the mammal from which the cells originated, and wherein said transduced cells are suitable for introduction into a mammal. Nor does Crabtree suggest administering the claimed divalent ligand to the treated mammal.

Rather, Crabtree refers to numerous possible receptor-ligand choices, or otherwise refers to a new technology or general approach that seemed to be a promising field of experimentation, but gives only general guidance as to the particular form of the claimed invention or how to achieve it.

Hence, the combination of Capon, Blau and Crabtree is deficient, for the reasons expressed above, in supporting a § 103 rejection. Indeed, the combination fails to provide a “combinati[on of] previously known elements.” *KSR Int'l Co v. Teleflex Inc.*, No. 04-1350 (April 30, 2007) at 14, 15. In summary, comparing the cited references in combination to the claimed invention, it

is clear that claimed invention reflects an advancement and “real innovation.” *KSR Int'l*, at 15.
Applicants respectfully request that the § 103 rejection be withdrawn.

CONCLUSION

Applicants respectfully request reconsideration of this application and allowance of the pending claims in view of the above remarks.

Except for issue fees payable under 37 C.F.R. §1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. §§1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0310. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 C.F.R. §1.136(a)(3).

Respectfully submitted,

Date: March 26, 2008 /Mary S. Webster, Reg. No. 37,156/
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Biochemical and biological characterization of lymphocyte regulatory molecules. V. Identification of an interleukin 2-producing human leukemia T cell line.

Gillis S, Watson J.

To isolate a stable tumor cell line capable of producing human interleukin 2 (IL-2; formerly referred to as T cell growth factor), 16 human T and B leukemia cell lines were screened for constitutive and mitogen-stimulated IL-2 production. We found that the T cell leukemia line designated Jurkat-FHCRC produced > 200 U/ml of IL-2 activity after a 24-h stimulation with T cell mitogens. Peak mitogen-induced IL-2 activity was found in supernates harvested from 24-h Jurkat-FHCRC cell cultures stimulated with either 1% phytohemagglutinin or 20 microgram/ml concanavalin A. Addition of the fatty acid derivative phorbol myristate acetate to mitogen-stimulated cultures increased Jurkat-FHCRC IL-2 production to concentrations > 400 U/ml. IL-2 activity observed in such cases represented between 100-300 times that produced in conventional cultures of mitogen- or alloantigen-stimulated normal human peripheral blood or splenic lymphocytes. Jurkat-FHCRC-derived conditioned medium demonstrated equal capacity to promote the sustained in vitro proliferation of either murine or human activated T cell lines confirming the ability of Jurkat-FHCRC cells to produce human IL-2. These studies identify a new source of human IL-2 and establish a valuable reagent for the isolation and further molecular characterization of this immunoregulatory molecule.

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GENES

Benjamin Lewin

Oxford New York Tokyo
Oxford University Press
1997

CHAPTER 37

Oncogenes and cancer

A major feature of all higher eukaryotes is the defined life span of the organism, a property that extends to the individual somatic cells, whose growth and division are highly regulated. A notable exception is provided by cancer cells, which arise as variants that have lost their usual growth control. Their ability to grow in inappropriate locations or to propagate indefinitely may be lethal for the individual organism in which they occur.

Three types of changes that occur when a cell becomes tumorigenic are summarized in Figure 37.1:

- ◆ **Immortalization** describes the property of indefinite growth (without any other changes in the phenotype necessarily occurring).
- ◆ **Transformation** describes the failure to observe the normal constraints of growth; for example, transformed cells become independent of factors usually needed for cell growth.
- ◆ **Metastasis** describes the stage at which the cancer cell gains the ability to invade normal tissue, so that it can move away from the tissue of origin and establish a new colony elsewhere in the body.

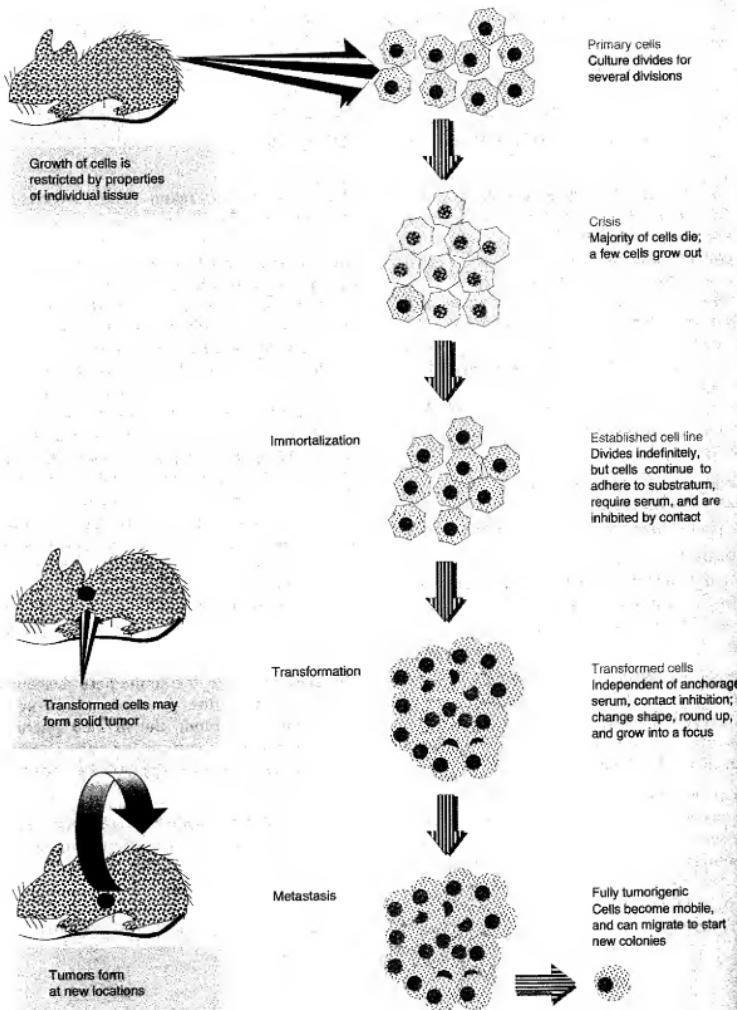
To characterize the aberrant events that enable cells to bypass normal control and generate tumors, we need to compare the growth characteristics of normal and transformed cells *in vitro*. Transformed cells can be grown readily, but it is much more difficult to grow their normal counterparts.

When cells are taken from a vertebrate organism and placed in culture, they grow for several divisions, but then enter a senescent stage, in which growth ceases. This is followed by a crisis, in which most of the cells die. The survivors that emerge are capable of dividing indefinitely, but their properties have changed in the act of emerging from crisis. The nature of crisis is poorly defined, and we do not understand the molecular changes that adapt a cell to growth in culture, but in principle this comprises the process of immortalization. (The features of crisis depend on both the species and tissue. Typically mouse cells pass through crisis at ~12 generations. Human cells enter crisis at ~40 generations, although it is rare for human cells to emerge from it, and only some types of human cells in fact can do so.)

The limitation of the life span of most cells by crisis restricts us to two options in studying nontransformed cells, neither entirely satisfactory:

- ◆ **Primary cells** are the immediate descendants of cells taken directly from the organism. They faithfully mimic the *in vivo* phenotype, but in most cases survive for only a relatively short period, because the culture dies out at crisis.
- ◆ Cells that have passed through crisis become **established** to form a (nontumorigenic) cell line. They can be perpetuated indefinitely, but their properties have changed in passing through crisis, and may indeed continue to change during adaptation to culture. These

Figure 37.1 Overview: three types of properties distinguish a cancer cell from a normal cell. Sequential changes in cultured cells can be correlated with changes in tumorigenicity.



changes may partly resemble those involved in tumor formation, which reduces the usefulness of the cells.

An established cell line by definition has become immortalized, but usually is not tumorigenic. Nontumorigenic established cell lines display characteristic features similar to those of primary cultures, often including:

- ◆ **Anchorage dependence**—a solid or firm surface is needed for the cells to attach to.
- ◆ **Serum (or growth factor) dependence**—serum is needed to provide essential growth factors.
- ◆ **Density-dependent inhibition**—cells grow only to a limited density, because growth is inhibited, perhaps by processes involving cell-cell contacts.
- ◆ **Cytoskeletal organization**—cells are flat and extended on the surface on which they are growing, and have a typical elongated network of stress fibers (consisting of actin filaments).

The consequence of these properties is that the cells grow as a **monolayer** (that is, a layer one cell thick) on a substratum.

These properties provide parameters by which the normality of the cell may be judged. Of course, any established cell line provides only an approximation of *in vivo* control. The need for caution in analyzing the genetic basis for growth control in such lines is emphasized by the fact that almost always they suffer changes in the chromosome complement and are not true diploids. A cell whose chromosomal constitution has changed from the true diploid is said to be **aneuploid**.

Cells cultured from tumors instead of from normal tissues show changes in some or all of these properties. They are said to be **transformed**. A transformed cell grows in a much less restricted manner: usually it does not need to attach to a solid surface (so that individual cells "round-up" instead of spreading out), has reduced serum-dependence, piles up into a thick mass of cells (called a **focus**) instead of growing as a surface monolayer, and may induce tumors when injected into appropriate

test animals. Figure 37.2 compares a "normal" fibroblast growing in culture with a "transformed" variant.

It would be naive to suppose that there is a uniform basis for cancer cell formation; many types of changes in the cellular constitution confer the ability to form a tumor. However, the joint changes of immortalization and transformation of cells in culture provide a paradigm for the formation of animal tumors. By comparing transformed cell lines with normal cells, we hope to identify the genetic basis for tumor formation and also to understand the phenotypic processes that are involved in the conversion.

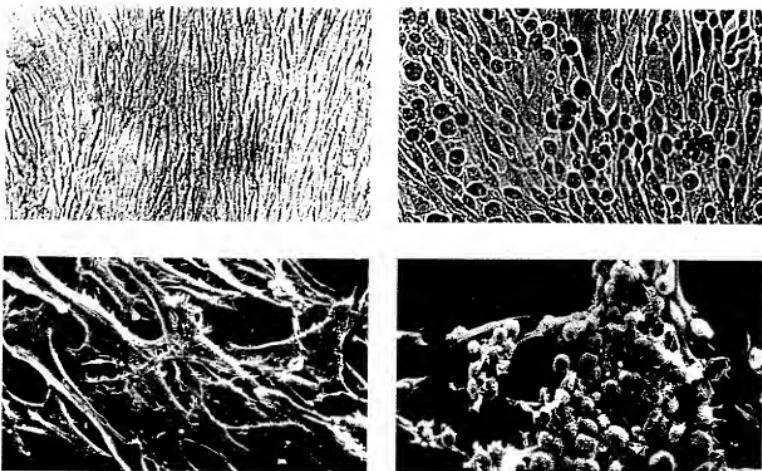
Certain events convert normal cells into transformed cells, and provide models for the processes involved in tumor formation. Usually multiple genetic changes are necessary to create a cancer; and sometimes tumors gain increased virulence as the result of a progressive series of changes. The incidence of human cancers with age suggests that typically 6–7 events are required over a span of 20–40 years to induce a cancer. In certain (rare) cases, propensity to cancer is inherited as a Mendelian trait, implying that a single genetic change is an important or necessary component (although other changes are also necessary).

A variety of agents increase the frequency with which cells (or animals) are converted to the transformed condition; they are said to be **carcinogenic**. Sometimes these carcinogens are divided into those that "initiate" and those that "promote" tumor formation, implying the existence of different stages in cancer development. Carcinogens may cause epigenetic changes or (more often) may act, directly or indirectly, to change the genotype of the cell.

There are two classes of genes in which mutations cause transformation:

- ◆ **Oncogenes** were initially identified as genes carried by viruses that cause transformation of their target cells. A major class of the viral oncogenes have cellular counterparts that are involved in normal cell functions. The cellular genes are called **proto-oncogenes**, and in certain cases their mutation or aberrant activation in the cell is associated with tumor

Figure 37.2 Normal fibroblasts grow as a layer of flat, spread-out cells, whereas transformed fibroblasts are rounded up and grow in cell masses. The cultures on the left contain normal cells, those on the right contain transformed cells. The top views are by conventional microscopy, the bottom by scanning electron microscopy. Photographs kindly provided by Hidesaburo Hanafusa and J. Michael Bishop.



formation. About 100 oncogenes have been identified. The oncogenes fall into several groups, representing different types of activities ranging from transmembrane proteins to transcription factors, and the definition of these functions may therefore lead to an understanding of the types of changes that are involved in tumor formation. The generation of an oncogene represents a gain-of-function in which a cellular proto-oncogene is inappropriately activated. This can involve a mutational change in the protein, or constitutive activation, over-expression, or failure to turn off expression at the appropriate time.

- ◆ Tumor suppressors are detected by deletions (or other inactivating mutations) that are

tumorigenic. The most compelling evidence for their nature is provided by certain hereditary cancers, in which patients with the disease develop tumors that have lost both alleles, and therefore lack an active gene. There is also now evidence that changes in these genes may be associated with the progression of a wide range of cancers. About 10 tumor suppressors are known at present. They represent loss-of-function in genes that usually impose some constraint on the cell cycle or cell growth; the release of the constraint is tumorigenic.

In the first part of this chapter, we consider how these two classes of genes are identified, and we ask how oncogenes are activated, and

how tumor suppressors are inactivated. Then we consider the molecular basis for these events, and how oncogenes are connected into

pathways that extend from signal transduction at the cell surface to activation of transcription factors in the nucleus.

Transforming viruses carry oncogenes

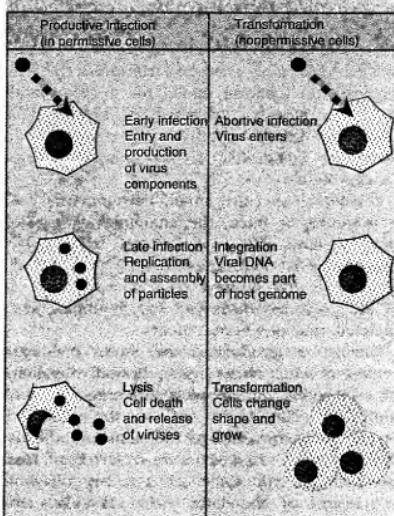
Transformation may occur spontaneously, may be caused by certain chemical agents, and, most notably, may result from infection with **tumor viruses**. There are many classes of tumor viruses, including both DNA and RNA viruses, and they occur widely in the avian and animal kingdoms.

The transforming activity of a tumor virus resides in a particular gene or genes carried in the viral genome. Oncogenes were given their name by virtue of their ability to convert cells to a tumorigenic (or oncogenic) state. An oncogene initiates a series of events that is executed by cellular proteins. In effect, the virus throws a regulatory switch that changes the growth properties of its target cell. Table 37.1 summarizes the general properties of the major classes of transforming viruses. The oncogenes carried by the polyoma and adenoviruses specify proteins that inactivate tumor suppressors, so their action in part mimics loss-of-function of the tumor suppressors. The oncogenes carried by retroviruses are derived from cellular genes and therefore may mimic the behavior of gain-of-function mutations in animal proto-oncogenes.

Polyomaviruses and adenoviruses have been isolated from a variety of mammals. Although perpetuated in the wild in a single species, a virus may be able to grow in culture on a variety of cells from different species. The response of a cell to infection depends on its species and phenotype and falls into one of two classes, as illustrated in Figure 37.3:

- ◆ **Permissive cells** are productively infected. The virus proceeds through a lytic cycle that is divided into the usual early and late stages. The cycle ends with release of progeny viruses and (ultimately) cell death.

Figure 37.3 Permissive cells are productively infected by a DNA tumor virus that enters the lytic cycle, while nonpermissive cells are transformed to change their phenotype.



- ◆ **Nonpermissive cells** cannot be productively infected, and viral replication is abortive. Some of the infected cells are transformed; in this case, the phenotype of the individual cell changes and the culture is perpetuated in an unrestrained manner.